CHROMOSOMES OF ARTIFICIALLY ACTIVATED EGGS OF URECHIS

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The eggs of *Urechis* that cleave and develop as a result of activation by dilute sea water have been previously shown (Tyler, 1931*a*) to be those which extrude no polar bodies. It would appear then that the embryos produced by such eggs might be tetraploid, diploid, or haploid, depending upon the behaviour of the chromosomes during the first two nuclear divisions. A cytological investigation of such eggs shows that the embryos are diploid in chromosome number, and that only one maturation division occurs.

The preparations were made by a method used by Karl Bělař and similar to that described by him (1928). It consists of joining and later separating two cover-slips, one containing a drop of eggs and the other a drop of fixing fluid. The eggs are flattened to any desired extent and stick to the cover-slips, which can be handled in the same manner as slides containing sectioned material.

Two types of eggs are produced as a result of activation with dilute sea water (Tyler, 1931*a*). In one type the initial behaviour is identical with that of the normally fertilized eggs, two polar bodies are produced but none of the eggs divide. In the other type the initial behaviour is quite different from that of normally fertilized eggs; no polar bodies are produced but practically all the eggs of this type divide and develop.

In making the cytological preparations of the eggs of the first type, use was made of the fact, previously reported (Tyler, 1931*b*), that an inverse relation exists between the total percentage of activation and the percentage of cleavage. Thus treatments giving 100 per cent activation produce only eggs of the first type which do not divide. For preparations of the type which does not extrude polar bodies, the eggs had to be isolated from dishes containing also unactivated eggs and activated eggs of the first type. These eggs can be readily distinguished at an early stage and can be removed for cytological preparations before the time at which the first polar body appears in the eggs of the first type.

The counts of chromosome number were generally made from polar views of anaphase groups inasmuch as precociously divided chromosomes in metaphase might cause difficulty.

The Eggs That Produce Polar Bodies

The behaviour of the chromosomes in the maturation division of the artificially activated eggs that extrude two polar bodies is identical with that of the normally fertilized eggs. The normal diploid number in *Urechis* is most probably thirty-six chromosomes and the haploid number eighteen. The variability in the chromosome numbers shown in the tables is undoubtedly due to errors in counting. The artificially activated eggs (last section of Table I) show the haploid number of

Table I

FIRST CLEAVAGE OF NORMAL FERTILIZED EGGS		FIRST POLAR DIVISION OF NORMAL FERTILIZED EGGS		FIRST POLAR DIVISION OF PARTHENOGENETIC EGGS	
Chromosome Number	Number of Groups	Chromosome Number	Number of Groups	Chromosome Number	Number of Groups
33	4	15	3	14	2
34	6	16	2	16	5
35	3	17	8	17	10
36	7	18	8	18	6
37	2	19	1	19	1

chromosomes at the first maturation division. The second polar division also occurs normally and the egg is left with the haploid number of chromosomes, which form a nucleus and move into the center of the egg. A large monaster then forms at about fifteen minutes after the extrusion of the second polar body and the chromosomes distribute themselves irregularly about the astral rays. The monaster disappears and a vesicular nucleus is formed about ten minutes later. The monaster then reappears about twenty minutes later and a larger and variable number of chromosomes are seen. The monaster may disappear and reappear a third time. This behaviour is essentially similar to that described by Herlant (1918) in the sea-urchin for eggs activated by butyric acid. The failure of the eggs of this type to divide appears then to be due to the failure to form an amphiaster.

The Eggs That Divide

In the eggs of this type the germinal vesicle breaks down and tetrad chromosomes appear at about twenty to twenty-five minutes after treatment. At this time the first polar spindle appears in the control eggs. But no spindle is seen in the eggs of this type and at about ten to twenty minutes later the tetrads each form a small vesicular karyomere. The

nucleolus generally persists as such throughout this time and about seventeen or eighteen karyomeres may be seen distributed throughout the egg. Later a single large nucleus is formed apparently by the fusion of the karyomeres. The nucleolus remains intact and is seen within the nucleus. This nucleus is generally about two-thirds of the size of the original germinal vesicle and has a granular appearance similar to that of the cytoplasm. The eggs remain in this condition for about an hour, after which the first cleavage spindle appears.

TABLE II

Parthenogenetic Eggs

FIRST CLEAVAGE		SECOND CLEAVAGE		THIRD CLEAVAGE	
Chromosome Number	Number of Groups	Chromosome Number	Number of Groups	Chromosome Number	Number of Groups
13	1	32	2	32	5
15	4	33	2	33	7
16	8	34	3	34	6
17	5	35	8	35	4
18	5	36	4	36	5
		38	1	37	2
				38	2
				40	1
		45	1	13	2
		46	1	14	2
		48	2	15	2
		50	1	16	4
		53	2	18	5
		54	1	20	1

An attempt was made to determine whether any division of the chromosomes occurred prior to the first cleavage, and whether fusion of the egg nucleus with a submerged polar body nucleus such as described by Morris (1917) in *Cumingia,* occurred in *Urechis.* The evidence shows that such behaviour does not occur in *Urechis.* It is possible to determine this point with some certainty in *Urechis* inasmuch as the eggs which are to divide remain indented until just before the first cleavage. The indented eggs were therefore preserved at close intervals up to that time. No division figures or fusion of two nuclei were observed. Furthermore, the chromosomes on the first cleavage spindle have the appearance of tetrads and are eighteen in number. In anaphase they open out as typical dyads.

It appears then that the first cleavage spindle is identical with the first polar spindle as far as the chromosomes are concerned, and the first division may be considered a maturation division.

At the second cleavage the diploid number of chromosomes is usually seen. This is the case with the twenty anaphase chromosome groups of the five eggs listed in Table II. The chromosomes of these groups occur in more or less closely associated pairs. This means that the chromosomes had divided previous to this division. In other words the second cleavage is a mitotic rather than maturation and the diploid number of chromosomes is retained. In two eggs listed in Table II eight anaphase groups gave chromosome counts approximating fifty-four,

TABLE III

Parthenogentic Eggs

FOURTH CLEAVAGE		EMBRYOS	
Chromosome Number	Number of Groups	Chromosome Number	Number of Groups
31	2	28	2
32	6	29	1
33	8	30	4
34	7	32	6
35	6	33	3
36	10	34	7
37	4	35	2
38	3	36	3
39	1	38	2
40	1		

the triploid number. The origin of such chromosome groups was not determined, and they were not encountered in the slides of the later stages.

In the anaphase of the third cleavage the diploid number is again usually seen. Two eggs were obtained, however, in which the chromosomes were of the haploid number. These must have arisen by the occurrence of both maturation divisions in the first two cleavages of the egg. The haploid number was not obtained again in the later stages of other eggs studied.

The chromosome counts at the fourth cleavage of the egg, Table III, again approximated thirty-six, the diploid number. For the embryos, normal top-swimmers were isolated and preserved in the usual manner. Counts on fifteen pairs of anaphase groups (Table III) showed that the diploid number is present.

The failure of the artificially activated egg that extrudes polar bodies to divide may be attributed to its retaining only the haploid number of chromosomes or to the possession of only the inner central body of the second polar spindle which is incapable of forming an amphiaster. The former is an unlikely assumption inasmuch as some haploid cleavage has been obtained in *Urechis*. But if the interpretation is based upon the behaviour of the central bodies,[1] it is difficult to see why the cleavage of those eggs that produce no polar bodies should go beyond the four-cell stage. At this stage the centrosomes should be equivalent to the three that would have gone into the polar bodies and the one that remains in the egg. It might appear then that when the polar body central bodies come to lie within the egg cytoplasm they are capable of forming amphiasters. It may be pointed out in this connection that the first polar body in *Urechis* may or may not divide. Correspondingly in the artificially activated eggs, one of the cells of the two-cell stage often fails to divide. Similarly in the four-cell stage one of the cells often fails to divide corresponding to the egg cell that receives the inner central body of the second polar spindle. This again may be interpreted to mean that the first polar spindle is used for the first division. However, in a large number of cases all four cells divide, and since no accessory asters have been observed in these eggs, it appears that the inner central body of the second polar spindle has regained the ability to form an amphiaster.

Summary

1. The embryos resulting from the artificial activation of *Urechis* eggs are diploid in chromosome number.

2. The diploid number is apparently obtained by the utilization of the first polar spindle for the first cleavage and the substitution of a mitotic division for the second maturation division.

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[1] This discussion is based on the assumption of the genetic continuity of the centrosomes to which there no longer appears to be serious objection.

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